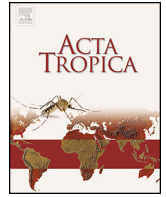




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Point-source and area-wide field studies of pyriproxyfen autodissemination against urban container-inhabiting mosquitoes



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ABSTRACT

Autodissemination of insecticides is a novel strategy for mosquito management. We tested if contaminated *Aedes albopictus* (Skuse) mosquitoes from a small area treated with commercial formulations (79 gm a.i. pyriproxyfen/ha) using conventional techniques, would disseminate pyriproxyfen over a wider area. Pyriproxyfen showed $LC_{50} = 0.012$ ppb for *Ae. albopictus*. Direct treatment and autodissemination efficacy was measured as a pupal mortality by conducting *Ae. albopictus* larval bioassay. A tire pile ($n = 100$ tires) treated by backpack sprayer as a point-source treatment showed higher pupal mortality in 2010 (60.8% for week 0–6) than in 2011 (38.3% for week 0–6). The sentinel containers placed for autodissemination in four compass directions out to 200–400 m from the tire pile showed 15.8% pupal mortality (week 1–6) in the first year, and 1.4% pupal mortality in the second year. No significant difference was detected among the distances and direction for pupal mortality. In area-wide treatments, vegetation was sprayed in checkerboard pattern (3.7% of 105 ha) using backpack sprayer in 2010 and in strips (24.8% of 94 ha) using truck-mounted ultra-low volume (ULV) sprayer in 2011. In both years, the area-wide direct treatment efficacy was lower (30.3% during 2010 and 5.3% in 2011) than point-source treatments. Autodissemination in area-wide plots was higher in 2010 (10.3%) than 2011 (2.9%). However, area-wide treatments were ineffective on field populations of *Ae. albopictus* as monitored by using BGS traps. We found accumulation of pyriproxyfen in the week 6 autodissemination containers in both experiments. The differences in autodissemination in 2010 and 2011 can be attributed to higher rainfall in the second year that may have eroded the pyriproxyfen from treatment surfaces and sentinel containers. Our study shows that ULV surface treatments of conventional formulation do not work for autodissemination. The effectiveness of pyriproxyfen in autodissemination may be improved by developing specific formulations to treat vegetation and tires that can load high doses on mosquitoes.

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1. Introduction

Pyriproxyfen is a pyridine-based insect growth regulator (IGR) that acts as a juvenile hormone analog, overloading the insect endocrine system and disrupting the normal development of immature stages with lethal results (Ishaaya and Horowitz, 1992). Pyriproxyfen is also considered a 'reduced-risk pesticide' or 'unlikely to present acute hazard' that is virtually non-toxic to

birds or animals and is neither carcinogenic or genotoxic and can be safely added to drinking water for mosquito control at a concentration of 0.01 ppm (WHO, 2009). But because some aquatic invertebrates, notably flies and copepods (EPA, 2000), can be affected, direct application to natural water bodies is prohibited in the US.

The field evaluation of pyriproxyfen against mosquito larvae has shown longer persistence and high efficacy (Yapabandara and Curtis, 2002; Nayar et al., 2002; Vythilingam et al., 2005). Despite this exceptional persistence as a larvicide, studies indicate a low potential for the development of resistance. For example, even after *Culex quinquefasciatus* (Say) larvae were pressured with pyriproxyfen exposure for 17 generations, their susceptibility remained

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unchanged (Schaefer and Mulligan, 1991). Yet this chemical has seen almost no use against mosquitoes. We suspect that pyriproxyfen possesses unexploited potential as a mosquito larvicide, particularly in regards to new control approaches such as autodissemination.

Autodissemination is a novel concept that has abruptly altered the mostly academic interest in pyriproxyfen for mosquito control. First demonstrated in laboratory trials by Itoh (1994) and subsequently confirmed by Chism and Apperson (2003), Devine et al. (2009) demonstrated in field experiments in Peru that gravid female *Aedes aegypti* (L.) contaminated with pyriproxyfen transfer lethal concentrations to larval habitats. Devine and his coworkers demonstrated pyriproxyfen autodissemination as a promising new approach to manage container-breeding mosquitoes. In the Devine et al. (2009) study, dissemination stations lined with dusted (crushed pyriproxyfen granules) cloth were used for autodissemination against sentinel ovicups holding *Ae. aegypti* larvae, which produced 42–98% inhibition of adult emergence. Recently, Caputo et al. (2012) achieved considerable dissemination of pyriproxyfen from a station coated with powdered granules under field conditions. Thus, high coverage of larval habitats was achieved by treating a small area with a minute amount of the active ingredient.

Our study was designed to build on the pyriproxyfen autodissemination work of earlier researchers via operational research in which this simple concept might be utilized for area-wide control using conventional insecticide formulations and equipment. Foremost, our goal was to develop a practical application strategy by testing a commercially available product in the USA and delivered via conventional spray equipment by local mosquito control personnel. Second, we aimed to extend the autodissemination concept to additional mosquito species in the field, specifically the important container-inhabiting species *Ae. albopictus*. This mosquito is a peridomestic vector of dengue and chikungunya (Gratz, 2004) with diurnal and exophagic activity (Hawley, 1988) that reduces the outdoor activities of children (Worobey et al., 2013). Similar to *Ae. aegypti*, this container-inhabiting mosquito also exhibits 'skip' oviposition behavior in which multiple larval habitats are visited by the gravid female (Trexler et al., 1998), resulting in increased opportunities for chemical dispersal. Third, whereas Devine et al. (2009) deployed 'stations' to disseminate pyriproxyfen, we conducted broadcast sprays to adult resting and larval habitats. The central hypothesis was that gravid females contaminated either directly from the spray or indirectly by contacting contaminated surfaces would transfer pyriproxyfen to new larval habitats. We tested our hypothesis using commercial product of pyriproxyfen with conventional sprayers in an area-wide treatment of vegetation and a point-source treatment of a tire pile to know the direct impact and autodissemination efficacy over the time and autodissemination distance. Our study showed autodissemination of pyriproxyfen from treated sites to untreated sentinel oviposition sites under field conditions in area-wide and point-source treatment experiments, and pyriproxyfen can be delivered by mosquitoes up to 200 m distances.

2. Materials and methods

2.1. Test chemical and sprayer

We used a commercial pyriproxyfen product labeled for mosquito control in the U.S. (NyGuard® IGR concentrate -MGK® Corp., Minneapolis, MN, USA) containing 10% pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine), formulated as an emulsifiable concentrate. In all point-source as well as area-wide field treatments, pyriproxyfen was applied as per manufacturer's recommendation (789.23 mL/ha).

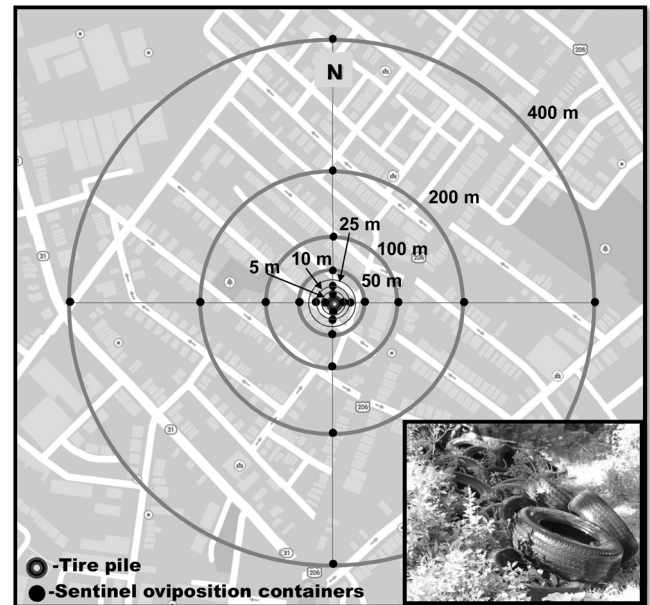


Fig. 1. Point-source treatment showing direct treatment of a tire pile (at the center) using a backpack sprayer, and autodissemination experiment setup at Trenton, NJ, USA. Four directional transects further show the locations of sentinel oviposition containers positioned from 5 to 200 m in 2010 and 25–400 m in 2011 to detect the extent of autodissemination distance. Inset shows tire pile consisting of >100 discarded automobile tires. (Map not to scale).

Stihl SR 420 backpack sprayers (Andreas Stihl Ag & Co. KG, Waiblingen, Germany) equipped with a mist blower conversion kit and tapered baffle screen was used for point-source treatment in 2010 and 2011 and for area-wide treatment in 2010. For point-source applications, the flow rate was 0.86 L/min at setting 3, which provided 84.6 μ m VMD (volume median diameter) droplets. For area-wide field applications, the flow rate was 0.44 L/min (setting 2), which provided 74.7 μ m VMD droplets. The higher setting for point-source applications was to enhance droplet penetration into stacked tires, whereas the lower setting for area-wide applications was intended to improve coating of surface areas to maximize mosquito–insecticide contact.

During 2011, area-wide pyriproxyfen application was made in a strip pattern with a truck-mounted single nozzle Cougar® ultra-low volume (ULV) cold aerosol sprayer (Clarke Mosquito Control, Roselle, IL). For best results, pyriproxyfen was applied from a vehicle moving at 8 Km/h, approximately perpendicular to the wind direction, using a swath width of 91.4 m. Spray equipment was adjusted for the VMD as 25–50 microns ($25 \mu \leq Dv0.5 \leq 50 \mu$) and 90% of the droplets were below 80 microns ($50 \mu \leq Dv0.9 \leq 80 \mu$). Recognizing label restrictions, a 50 m buffer was provided to exclude natural water bodies from the treatment area. Only a single application was conducted for the experiment.

2.2. Point-source treatment

2.2.1. Study area

Dissemination of the chemical from a single localized treatment to larval habitats was tested by creating a point-source infestation. In mid-June 2010, a tire pile was constructed at the treatment site by placing 100 discarded vehicle tires in a mound up to a 1.2 m height and encompassing an area of 20.9 m² (Fig. 1). Only rainwater was available to provide larval habitat in the tires. Subsequent sampling demonstrated that the tires were soon colonized by *Ae. albopictus*. The point source treatment site was located in an abandoned lot (40.229130 lat., -74.764184 long.) within a residential area in Trenton, NJ. The control site was located in a commercial

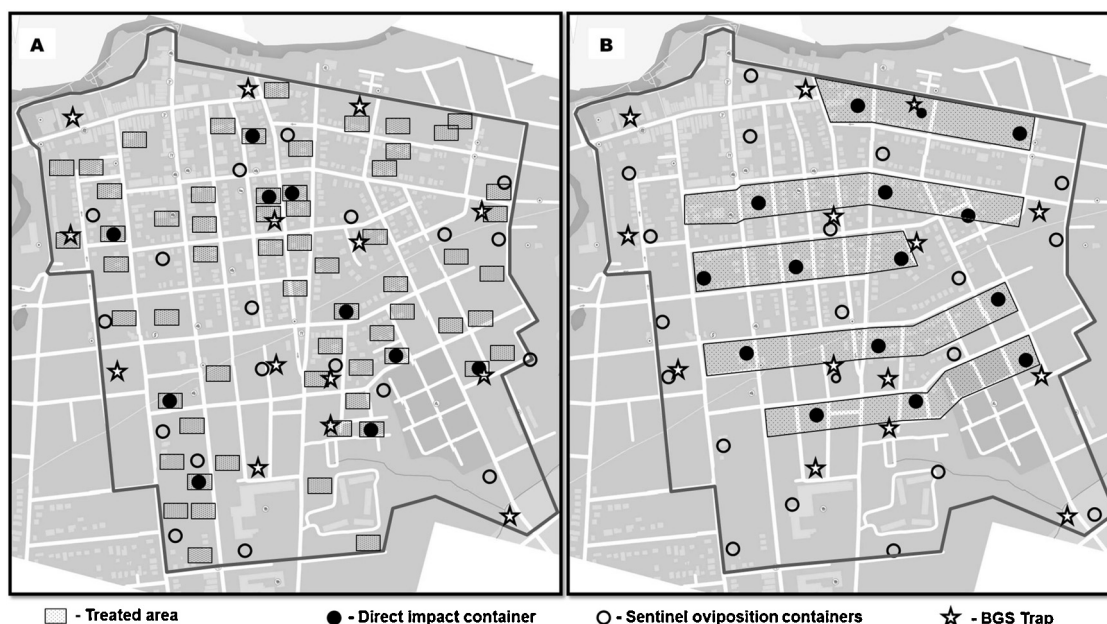


Fig. 2. Map showing the location of the area-wide treatment in Keyport, Monmouth County, NJ, USA. (A) Locations of parcels treated with backpack sprayer, direct treatment containers, sentinel autodissemination containers, and BGS traps in 2010, (B) locations of truck-mounted ULV treatment strips, direct treatment containers, autodissemination containers, and BGS traps in 2011. (Maps not to scale).

area (automotive repair shop at 40.234807 lat., -74.744401 long.) and separated from the treatment site by 1.9 km. The locations are densely populated urban localities (4,336 people/km²) (<http://quickfacts.census.gov/qfd/states/34/3474000.html>). The tire pile was treated with the maximum label rate of NyGuard on 31 August 2010 when *Ae. albopictus* adult populations were high (>5 *Ae. albopictus*/BGS trap).

2.2.2. Direct impact

Direct impact of the pyriproxyfen was assessed by larval bioassay of water samples collected from ten tires (250 mL) at 0, 1, 2, 4 and 6 weeks after treatment. Tires were randomly selected, and if individual tires had insufficient water quantities, then multiple tires were sampled to reach an aggregate of 250 mL. To avoid probability of contamination with other insecticides, bioassays were done on 10 randomly selected tires before treatment. In 2011, contents inside of tires were cleared of organic debris, thoroughly washed with tap water. Then, tires were tested for any contamination of insecticide by larval bioassay of the water sampled from the tires before treatment.

2.2.3. Autodissemination efficacy

Pyriproxyfen autodissemination with the point-source treatment was assessed by running transects from the tire pile in four opposite compass directions (Fig. 1). A pair of plastic sentinel containers (green plastic cemetery cone vase Item# 05011, 365 mL capacity, 17.5 cm height with 7.5 cm stake, 7.5 cm top diam.; Factory Direct Craft, Springboro, OH, USA) were fixed at the back of parcels with full or partial shade from adjacent vegetation 2 h after treatment along each transect. In 2010, containers were placed at 5, 25, 50, 100, and 200 m distant from the tires. All containers were filled with 250 mL of oak leaf infusion water to attract oviposition-seeking females. Oak leaf infusion was prepared using 5 g of dry oak leaf per 8 L of tap water (Trexler et al., 1998) in 50 L trash cans. Oak leaves of *Quercus alba* (L.) and *Q. rubra* (L.) were used because this infusion elicits oviposition responses from container-inhabiting *Aedes* mosquitoes (Trexler et al., 1998). The infusion was allowed to ferment for one week before use and was discarded after two weeks.

One sentinel container was sampled (250 mL) at 1, 2 and 4 weeks post-treatment from each direction and distance and was refilled with the same quantity of uncontaminated oak leaf infusion. The second container was sampled on the last week. Based on the 2010 results, the distance between containers was expanded up to 400 m (25–400 m) in 2011. There was a group of five containers, one container for each week of sampling to avoid dilution by sampling and refilling. One container from each distance and direction was sampled 1, 2, 4 and 6 weeks post-treatment and transported to the laboratory for larval bioassays as described above. The control site contained five containers. The control samples were collected as described for treated samples.

2.3. Area-wide treatment

2.3.1. Study area

The area-wide treatment site was located in Keyport, NJ (40.2608 lat., -74.1204 long.) and measured 105 ha in area (Fig. 2), whereas, the control site was in Union Beach, NJ (40.2650 lat., -74.71026 long.) and spanned 181 ha. Both the treatment and the control sites encompassed more than 1000 parcels, with each parcel corresponding to a structure or a house (residential or commercial) and surrounding yard. The sites were similar in socioeconomics and middle class neighborhoods (Unlu et al., 2011), however, Keyport was more populated (2027.3 people/km²) than Union Beach (1384.7 people/km²). We initially intended to assess pyriproxyfen-based area-wide autodissemination by treating 25% of an urban field plot encompassing at least 1000 parcels in five broad strips. State regulators, however, citing a mosquito-specific label restriction on 'truck-mounted sprayers and wide area control' did not approve this plan in 2010. To meet the requirement against wide area control, we followed state and federal regulations that limit treatment to areas of less than four hectares. Backpack sprayers were used in place of truck-mounted equipment to treat the foliage-covered area of the parcels. We did not spray roads, sidewalks, driveways, patios and buildings in each parcel. The treatment plot measured 105 ha in area and 4 ha (3.7%), including 74 parcels, was treated with available foliar area (59 ha). The applications were made in a scattered or 'checkerboard' pattern on 11 and

12 August 2010 at the NyGuard label rate of 789.2 mL/ha, when adult *Ae. albopictus* populations were determined from BGS trap counts to be near peak density (Fig. 6A). To ensure that treated parcels were dispersed throughout the plot, the area was divided into four zones and similar number of applications were conducted in each zone. Because we proposed to treat more than ten acres using a vehicle-mounted sprayer in 2011, we obtained an experimental use permit (EUP) from US Environmental Protection Agency (#88144-EUP-1) for truck-mounted ULV spray of NyGuard (789.23 mL/ha) in 33% (30.99 ha) of the study area (93.88 ha) at Keyport, NJ, however, only 24.8% was plot treated into five strips alternate to untreated area instead of backpack sprayer (Fig. 2B). Pyriproxyfen was sprayed when density reached maximum peak (Fig. 6B). Three metrics were assessed: direct, autodissemination, and adult population impact.

2.3.2. Direct impact

Direct impact of the pyriproxyfen spray within treated parcels was estimated using larval bioassay of samples from the treated area. Four containers with 250 mL of oak leaf infusion water were placed in each of 10 treated and five control parcels immediately before treatment. The containers were clustered together in protected backyards near vegetation that provided full or partial shade. One container per parcel was recovered at 0 (2 h), 2, 4, and 6 weeks post-treatment and 250 mL water sampled into a deli cup (480 mL capacity) for larval bioassays.

2.3.3. Autodissemination

Autodissemination, that is pyriproxyfen migration outside of the treatment parcels, was assessed from sentinel containers placed inside untreated autodissemination areas 24 h post-treatment. Twenty untreated parcels were marked for sentinel container placements. In 2010, two containers were placed in a pair, one container from the pair was sampled weekly while the other container was undisturbed and sampled only during the week to determine accumulation of pyriproxyfen. However, five containers in a group were placed during 2011, one container for each week. Containers were filled with 250 mL oak leaf infusion as an oviposition attractant for *Ae. albopictus*. The container used for weekly sampling in 2010 was refilled after sampling. One sentinel container was sampled from each site on 1, 2, 4 and 6 weeks post-treatment, the water sample was transferred to a deli cup (480 mL) and tested for pyriproxyfen activity. At the control site, five pairs of containers were used for both the direct impact and autodissemination experiments. The first container was sampled according to the sampling schedule of direct impact and autodissemination experiments. The second control container was undisturbed, other than weekly water replenishment, until week 6 when the container was sampled and used as the control for the 6th week autodissemination container.

2.3.4. Adult populations

In area-wide experiments, adult mosquito population impact was assessed using BG Sentinel™ traps baited with BG-Lure which contains ammonia, lactic acid, and fatty acids (Biogents AG, Regensburg, Germany). The plots, treatment and control, were divided in to 100 m vertical and horizontal grids. Locations for trap placement were selected by overlaying a grid of approximately 300 m between traps (Fig. 2A and B). In the direct treatment and autodissemination plot at Keyport, 14 BGS traps were placed at marked locations in the month of June on weekly basis. Distance between BGS traps was according to the flight range of *Ae. albopictus* (Niebylski et al., 1994; Marini et al., 2010). Similar grid system was used to deploy BGS traps ($n=21$) in control plot at Union Beach at similar time of the treatment. Based on our preliminary data showing traps in exposed areas collected significantly fewer adults, we positioned traps in areas sheltered from direct wind and sunlight as well as from rain.

Efforts were made to place the trap as close as possible to the center of the each grid-dependent on homeowner's permission. Trap locations were recorded with GPS units. Traps were deployed for 24 h once per week. Mosquitoes were collected for identification. Male and female mosquitoes were pooled and retained.

2.4. Pyriproxyfen bioassays

2.4.1. Mosquito rearing

Larvae of *Ae. albopictus* were obtained from a colony established at the Center for Vector Biology, Rutgers University, originally collected from eggs in Mercer County, NJ, USA in 2008 and supplemented during 2009 and 2010. Mosquitoes were reared as described by Gaugler et al. (2012). Animals for blood feeding were cared for as per Animal Use Protocol #86-129 of Rutgers University, USA.

2.4.2. Susceptibility of laboratory *Aedes albopictus* populations to pyriproxyfen

Pyriproxyfen insecticidal activity was tested using standard WHO protocols (WHO, 2005) to establish LC_{50} and LC_{90} levels. A 1% stock pyriproxyfen solution was prepared from NyGuard emulsifiable formulation and used for serial dilutions to test six concentrations ranging from 0.001 to 0.5 ppb based on reports of Ali et al. (1995). Stock solution and serial dilutions were prepared using tap water and were discarded after each experiment. Each concentration and the control were tested in five replicates. Controls were not treated. Twenty 3rd instar *Ae. albopictus* were exposed in 200 mL water in a bioassay cup (480 mL capacity, 9 cm diam.) and were incubated at 26 °C and 16:8 L:D photoperiod. Twice a week, ground rat chow (30 mg/L) was provided as food, water lost due to evaporation was replenished, and dead and emerged individuals were removed and recorded until all the individuals were emerged as adult or dead. Incomplete emergence or adults with attached exuvia were recorded as dead pupa. Larval death was excluded from the analysis as >98.5% of test individuals died in the pupal stage as is characteristic for pyriproxyfen insect growth regulator (Suman et al., 2013). Experiments were repeated twice.

2.4.3. Activity of pyriproxyfen in field samples

All field samples of direct impact and autodissemination from the point-source and area-wide experiment were filtered using a paper towel to remove wild mosquito populations and organic debris. From each sample 200 mL water was used for pyriproxyfen activity determination into a bioassay cup (480 mL capacity and 9 cm diam. plastic containers with a screen-covered lid). If necessary, water volume was replenished to 200 mL by adding dechlorinated tap water during sampling. Twenty laboratory reared third instar *Ae. albopictus* were exposed to the field samples. Larval bioassay procedure was followed as described in Section 2.5.2. Pupal mortality was recorded to show pyriproxyfen activity in the field samples.

2.5. Meteorological data

Daily weather forecast of rainfall and temperature (min., max., and average) was obtained from Trenton Airport, Mercer County, NJ and Keyport, Monmouth County, NJ stations (<http://www.wunderground.com>). Daily average temperature and cumulative weekly rainfall were used to express meteorological conditions of the experimental areas. Data from both years 2010 and 2011 were compared using *T-test* ($p < 0.05$) for significant differences. To find out the impact of rainfall on pyriproxyfen efficacy, correlation analysis (r) was performed between cumulative rainfall

and pupal mortality of direct impacts from Keyport and Trenton during 2010 and 2011.

2.6. Statistical analysis

In all experiments, mortality in controls was adjusted with treatments using Abbott's formula (1925) (Abbott, 1925). LC_{50} and LC_{90} of pyriproxyfen for pupal mortality of laboratory populations was estimated using probit analysis (Finney, 1971) (PASW statistics version 18). One-way analysis of variance (ANOVA) was used to compare the different sampling weeks of direct as well as autodissemination for significant differences using Fisher's least significant difference (LSD) at $p < 0.05$. Pupal mortality was correlated with distance to determine autodissemination efficacy over the distances in point source treatment experiment. Adult mosquito density (male and female) collected with BGS traps in area-wide treatment and control areas were compared using a non-parametric analysis, Mann-Whitney (Wilcoxon) W test to compare medians ($p < 0.05$) and F -test to compare the standard deviation for fluctuation in mosquito density ($p < 0.05$). Data are presented as mean \pm SE unless otherwise mentioned.

3. Results

3.1. Susceptibility of laboratory *Aedes albopictus* to pyriproxyfen

NyGuard emulsifiable formulation of pyriproxyfen killed *Ae. albopictus* in pupal stage (98.5% individual died as pupae) when exposed as 3rd instars under laboratory bioassays at extremely low levels. The LC_{50} of pyriproxyfen was 0.012 ppb (95% FL = 0.009–0.015) and the LC_{90} was 0.29 ppb (95% FL = 0.183–0.531), with complete inhibition of adult emergence invariably obtained at 1 ppb (intercept = 1.774, $df = 4$, $\chi^2 = 2.63$, $p < 0.0001$).

3.2. Efficacy of pyriproxyfen in point-source treatment

3.2.1. Direct impact

The point-source treatment provided further evidence of the direct impact of pyriproxyfen on *Ae. albopictus*. Overall average pupal mortality over the 6 week study was $60.8 \pm 12.3\%$ in 2010 (Fig. 3A). Water collected from the treated tires immediately after the treatment resulted in $95.5 \pm 1.6\%$ pupal mortality. At one week post-treatment, tire water still retained a high level of efficacy showing $81 \pm 4.1\%$ pupal mortality that diminished significantly to $58.6 \pm 3.7\%$ at week 2 post-treatment and was further reduced to $32.8 \pm 6.8\%$ at week 6 ($df = 4$, $F = 39.6$, $p < 0.0001$; Fig. 3A). In 2011, direct impact showed lower overall pupal mortality ($38.3 \pm 17.9\%$) in comparison to 2010 (Fig. 3B). There was no significant difference between pupal mortality at week 0 ($83.1 \pm 6.2\%$) and week 1 ($76.5 \pm 7.7\%$) post-treatment but was reduced significantly to $31.3 \pm 9.7\%$ at week 2 post-treatment ($df = 3$, $F = 31.8$, $p < 0.0001$). Week 4 and 6 samples did not show any effect from the treatment (Fig. 3B). Control mortality was $0.6 \pm 0.3\%$ in 2010 and $0.4 \pm 0.3\%$ in 2011.

3.2.2. Autodissemination efficacy

Autodissemination of pyriproxyfen was detected in sentinel containers under field conditions. In 2010, overall pupal mortality was $15.8 \pm 1.8\%$ (Fig. 3A). Pupal mortality was $16.1 \pm 2.3\%$ at week 1 post-treatment and was consistent during week 2 and 4 ($df = 3$, $F = 2.92$, $p = 0.04$). The accumulation samples (week 0–4) showed higher pupal mortality ($20.8 \pm 2.2\%$) than those of other weeks (Fig. 3A). There was no difference among varying distances ($13.8 \pm 2.3\%$ to $17.3 \pm 1.2\%$ pupal mortality for 5–200 m) ($df = 4$, $F = 0.59$, $p = 0.67$) as well as the directions ($15.3 \pm 5.5\%$ to $16.7 \pm 2.8\%$ pupal mortality) for pupal mortality ($df = 3$, $F = 0.11$,

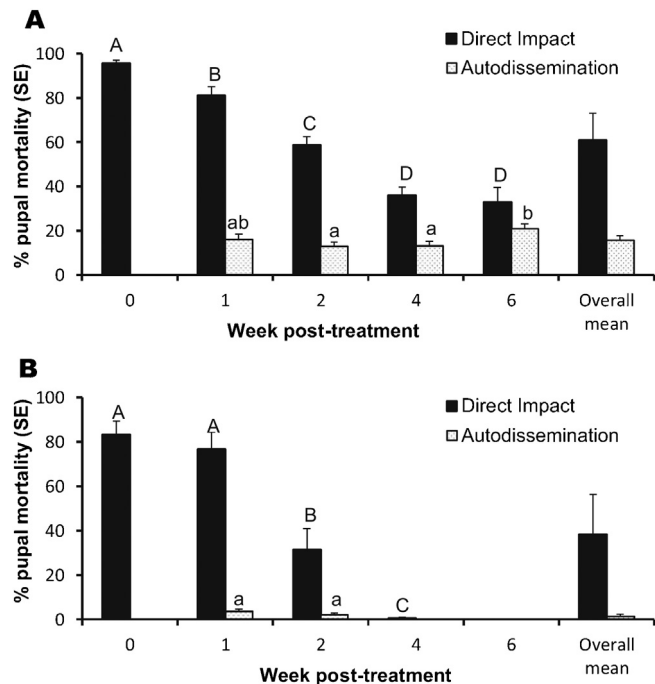


Fig. 3. Direct treatment and autodissemination efficacy of pyriproxyfen presented as percent pupal mortality of *Aedes albopictus* over time in point-source treatment at Trenton, NJ, USA during 2010 (A) and 2011 (B). Bars with similar letters do not differ significantly (One-way Anova, $p < 0.05$).

$p = 0.95$; Fig. 4). However, directional analysis of the pupal mortality with the distances showed that pupal mortality increased with distance in the north direction ($r = 0.9$), whereas, pupal mortality decreased with distance in the east ($r = -0.8$) (Fig. 4). In 2011, autodissemination efficacy was lower than that of 2010 (Fig. 3B). Pupal mortality was $3.6 \pm 1.0\%$ on week 1 and $2.0 \pm 0.9\%$ on week 2 post-treatment (t -test = 1.36, $p = 0.262$). There were no significant differences among the distances ($0.6 \pm 0.7\%$ to $2.4 \pm 1.0\%$ pupal mortality for 25–400 m) ($df = 4$, $F = 0.84$, $p = 0.52$) or as directions ($0.7 \pm 0.9\%$ to $2.8 \pm 1.4\%$ pupal mortality) ($df = 3$, $F = 2.86$, $p = 0.07$) for overall pupal mortality, similar to 2010. Pupal mortality of north, south, and west directions were positively correlated with distances, however, correlation was negative for the east (Fig. 4).

3.3. Area-wide treatment efficacy of pyriproxyfen

3.3.1. Direct impact

In the area-wide treatment, water from the containers placed within the treated parcels using backpack sprays showed higher efficacy than the ULV treated areas. Pupal mortality was $42.0 \pm 10.6\%$ at week 0 and declined to $17.0 \pm 4.8\%$ at week 6 post-treatment resulting as $30.3 \pm 5.5\%$ overall pupal mortality in 2010

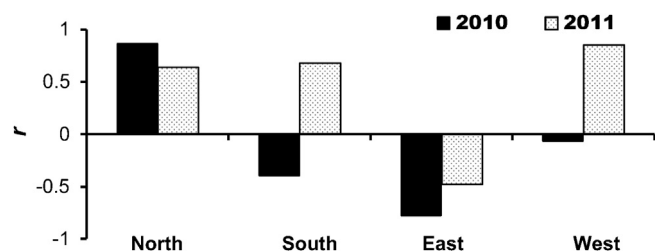


Fig. 4. Correlation analysis (r – correlation coefficient) between % pupal mortality of *Aedes albopictus* from autodissemination of pyriproxyfen and distance from various directions with the point-source treatment of tire pile, Trenton, NJ, USA during 2010 and 2011.

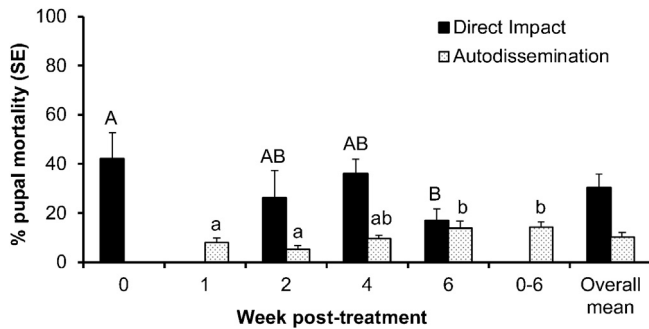


Fig. 5. Direct impact treatment and autodissemination efficacy of pyriproxyfen presented as pupal mortality of *Aedes albopictus* at different time intervals in area-wide treatment at Keyport, NJ, USA during 2010. Bars with same letters do not significantly differ (One-way Anova, $p < 0.05$).

($df = 3$, $F = 1.63$, $p = 0.19$; Fig. 5). In 2011, truck-mounted ULV spray in strips pattern covering 24.8% of the plot area resulted in $10.4 \pm 7.7\%$ pupal mortality two hours after treatment (Fig. 2B). Insecticidal activity declined by nearly one-half one week after treatment and reached $2.0 \pm 1.5\%$ at week 6. There was no significant difference among any week for pupal mortality ($df = 4$, $F = 0.61$, $p = 0.66$). Mortality in controls was $0.6 \pm 0.3\%$ in 2010 and $0.8 \pm 0.3\%$ in 2011.

3.3.2. Autodissemination efficacy

Area-wide treatment exhibited pyriproxyfen autodissemination using both backpack and truck-mounted ULV treatment strategies. In 2010, overall pupal mortality was $10.3 \pm 1.8\%$ which was $8.0 \pm 1.9\%$ and $14.0 \pm 2.8\%$ at week 1 and 6 post-treatments, respectively, showing an increase over time ($df = 4$, $F = 4.58$,

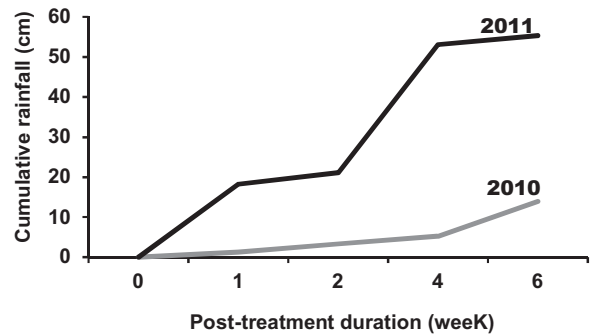


Fig. 7. Cumulative rainfall at experimental plots in NJ, USA during 2010 and 2011 (t test, $p < 0.01$).

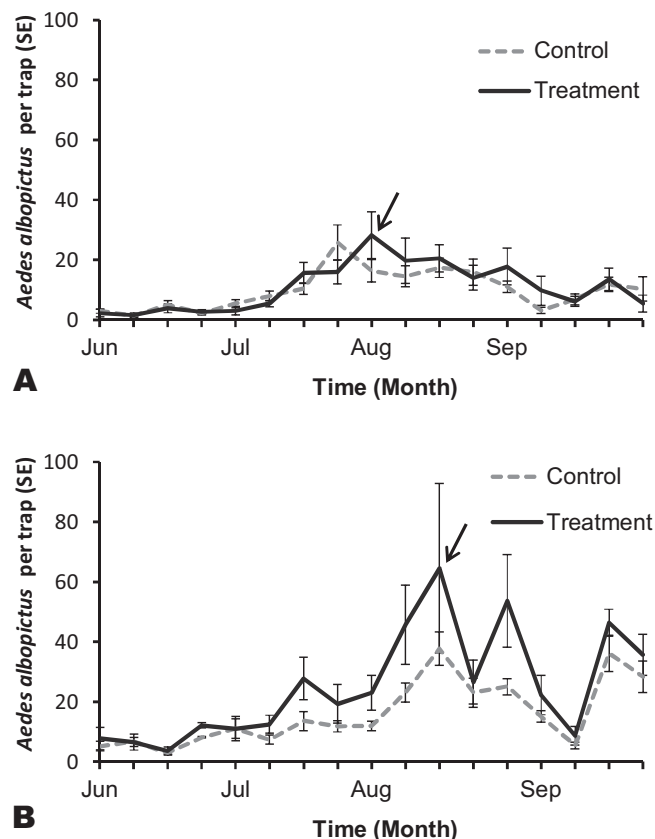
$p = 0.002$; Fig. 5). Efficacy of accumulation samples (week 0–6) was similar to week 6 ($df = 3$, $F = 3.22$, $p = 0.03$; Fig. 5). In 2011, autodissemination efficacy was lower ($2.9 \pm 1.1\%$) than in 2010.

3.4. Adult populations

In the treatment area, 15 and 16 mosquito species were collected using BGS traps during 2010 and 2011, respectively. Control plots showed 19 and 21 species during these same years. *Aedes albopictus* were the predominant species at both treatment (89.7% of 2,987 mosquitoes and 82.6% of 7049 mosquitoes) and control (70.4% of 6,378 mosquitoes and 60.6% of 5948 mosquitoes) sites during 2010 and 2011. *Culex pipiens* (L.) (1.7–2.0%), *Cx. restuans* (Theobald) (2.0–2.3%) and unidentified *Culex* spp. (4.5–11.4%) comprised the second major portion of collected mosquitoes. Other container-inhabiting mosquitoes were also collected, such as *Ae. japonicus* (Theobald) (0.2–0.3%), and *Ae. triseriatus* (Say) (0.4–0.5%), but at very low proportions. The population density of adult *Ae. albopictus* was 19.7 mosquitoes/trap during the week of treatment and increased slightly on week 1 (20.5 mosquito/trap) and declined gradually on weeks 2, 4, and 6 (6.18 mosquito/trap) in the treatment and autodissemination plots in 2010 (Fig. 6A). During 2011, mosquito density was higher (45.7 per trap) than the previous year during the treatment and increased to 64.6 mosquitoes per trap on week 1 and then declined gradually to 8.6 mosquitoes per trap on week 6 (Fig. 6B). No significant reduction in adult population density in the treatment area was observed in comparison to control areas during 2010 or 2011 ($p > 0.05$).

3.5. Meteorological conditions and effects of rainfall on direct treatment efficacy

During August 2010, rainfall amounts were below historic averages in New Jersey (1895–2009). The cumulative precipitation was 1.2, 3.3 and 5.3 cm on week 1, 2 and 4 post-treatments and reached 13.9 cm at week 6. Precipitation in 2011 was significantly higher than the previous year; week 1 post-treatment received 18.3 cm of precipitation (15 times more than the previous year) and 55.4 cm cumulative precipitation at week 6 post-treatment (3.9 times more than the previous year) (http://climate.rutgers.edu/stateclim_v1/data/njhistprecip.html) ($p = 0.012$; Fig. 7). Additionally, New Jersey experienced Hurricane Irene 2 week post-treatment (August 28, 2011) which delivered extremely heavy rainfall (15.2–20.3 cm) (<http://nj.usgs.gov/hazards/flood/flood1108/index.html>). The correlation between rainfall and direct impact pupal mortality was negative for both point-source treatment ($r = -0.828$ and -0.593) and area-wide treatment ($r = -0.828$ and -0.932) in 2010 and 2011, respectively. The average temperature during the experimental period in 2010 was 19.9°C which increased to 21.9°C in the second year ($p = 0.02$).



4. Discussion

Our study shows that treating a small area, vegetation and tires, with commercial pyriproxyfen formulation using conventional techniques results in pyriproxyfen autodissemination over a far wider area of larval habitats of container mosquitoes, although the efficacy was low and varied between the experiments.

Point-source treatment was conducted to determine efficacy in tires as well as a source of pyriproxyfen autodissemination. We achieved encouraging results that showed high overall pupal mortality from directly treated tires sampled during 2010, although mortality was lower in 2011. The best explanation for this difference is increased precipitation in 2011, which may have diluted pyriproxyfen in treated tires. In particular, Hurricane Irene may have flushed pyriproxyfen from the tires at week two, as no mortality was observed thereafter, whereas mortality was 32.8% by week 6 in 2010. This long term persistence may be attributed to other factors, such as adsorption of pyriproxyfen onto substrates particularly tires (Suman et al., 2013) and release over time can extend pyriproxyfen efficacy (Schaefer et al., 1988). Organic matter in automobile tires likely provides sites for pyriproxyfen adsorption during 2010. During 2011, debris and organic contents were washed out from the tires due to higher rainfall, which may have resulted in loss of insecticidal activity.

Our point-source treatment study showed that pyriproxyfen was disseminated to oviposition sites at least 200 m distant from the treated tire pile which is under flight range of *Ae. albopictus* (Niebylski et al., 1994; Marini et al., 2010). Albeit pupal mortality was low in autodissemination that can be attributed to low pyriproxyfen concentration in tires for autodissemination due to label rate for larvicide application. Tires are preferred oviposition and resting habitats for *Ae. albopictus* (Hawley, 1988) and provided an opportunity for use as a source for pyriproxyfen autodissemination. Autodissemination efficacy was 11 times higher in 2010 than 2011. August 2010 was the 3rd driest month ever after 1866 and there was no rainfall in 12 days post-treatment and one week pre-treatment, which did not generate competing oviposition habitats. Therefore our sentinel containers were primary oviposition sites for mosquitoes coming from the tire pile which resulted higher efficacy in 2010. Conversely, high rainfall in 2011 created competing oviposition sites, reducing direct impact and diluting the autodissemination containers.

Additionally, we found that autodissemination increased with distances in the north direction whereas it was reversed for the east direction. Correspondingly, a roadway to the north direction facilitated mosquitoes to fly for longer distances, whereas the east was blocked by housing and trees in the backyards that restricted direct mosquito flight. This suggests that information on directional effects on oviposition site selection due to geographical variations can be useful for designing control strategies against *Ae. albopictus*.

In area-wide direct treatments, vegetation was treated as peridomestic habitats with vegetation are suitable resting sites for *Ae. albopictus*. Direct treatments were made in backyard parcels with backpack sprayer in 2010 and in strip pattern with a truck-mounted ULV spray in 2011. Both treatments were considerably different, resulting for example in nearly six times better impact in 2010 than in 2011. It is likely that the ULV spray did not deliver enough pyriproxyfen to the containers, in spite of having significantly low lethal concentration ($LC_{50} = 0.012$ ppb). Contrary to it, Scott et al. (2013) recorded >75% inhibition of adult emergence (IE) in ULV treated containers at Florida that can be attributed to the large size containers (946 ml capacity) facilitating more ULV droplets intake in comparison to the present study. In our field trials, containers placed under the bushes or trees to mimic cryptic habitats of *Ae. albopictus* were obscured to ULV spray producing lower larvicidal efficacy than the reported by Scott et al. (2013).

We suggest backpack sprayers larvicide application in a localized infested area than over the broad area coverage of ULV sprays for container-inhabiting mosquitoes.

In our area-wide study, low autodissemination of pyriproxyfen was detected, perhaps due to less direct treatment area in comparison to the autodissemination area as described by Devine et al. (2009). Substantial direct treatment efficacy throughout the month during the first year confirms the availability of pyriproxyfen from where contaminated mosquitoes continuously transferred pyriproxyfen to sentinel containers. However, higher rainfall in 2011 washed out ULV treated vegetation and diluted sentinel containers resulting in inconsiderable efficacy of pyriproxyfen. It is evident from the study of Scott et al. (2013) that vegetation received significant amount of pyriproxyfen and obtained >85% IE from the water soaked with ULV treated leaves, however, did not measure pyriproxyfen residue on vegetation overtime. Study suggests the development of better pyriproxyfen formulations that can persist for longer time on vegetation for the autodissemination.

The success of mosquito control interventions is measured by population density. BGS traps have been used to estimate *Ae. albopictus* populations and showed greater sensitivity than ovitraps when populations are low (Fonseca et al., 2013). In our field trials, there was no reduction in *Ae. albopictus* populations in the areas treated with backpack or truck-mounted ULV sprayers. Ineffectiveness against adult populations may be because of (1) less direct treatment area, (2) low direct impact and autodissemination efficacy, (3) the cryptic habitats of *Ae. albopictus* that is difficult to penetrate by direct treatments, and (4) no population reduction immediately post-treatment due to slow mode of action of pyriproxyfen. The delayed reduction of current population peaks with low pupal mortality from small treated area was difficult to document. Moreover, the treated and untreated autodissemination areas were adjacent to each other allowing infiltration of mosquitoes between them which makes difficult to analyze effects of pyriproxyfen treatments on population density of these treated areas.

The properties of pyriproxyfen to adsorb onto substrates (Suman et al., 2013) helps in accumulation in sentinel containers making pyriproxyfen better larvicidal candidate for autodissemination technology. We found pyriproxyfen accumulation in containers exposed for longer durations in both sites of autodissemination at Trenton and Keyport, NJ which results in higher pupal mortality in accumulation containers than those containers exposed for week one or two. Accumulation in autodissemination was supported by several factors such as availability of pyriproxyfen in treated sites, mosquito population and low rainfall that can play important role in extending insecticidal effects on mosquitoes surviving in small habitats such as containers.

In conclusion, we detected autodissemination with point-source and area-wide treatments using commercial pyriproxyfen formulation via conventional sprayers. We also found that pyriproxyfen can be transferred at least 200 m from treated sites. Autodissemination efficacy is affected by several factors such as direct treatment efficacy, coverage of treated areas, treatment methods, geographical variations and rainfall. Our experiences lead us to an alternative strategy for the development of better autodissemination formulation and delivery method that can load high doses of pyriproxyfen on mosquitoes to transfer for long duration (Gaugler et al., 2012; Wang et al., 2013). In addition to autodissemination, direct treatment in the tire pile showed long term persistence of pyriproxyfen up to week 6 with a single application using backpack sprayer. We did not find ULV applications suitable for either the larval habitat treatment or autodissemination for container mosquitoes, however, Scott et al. (2013) achieved higher efficacy in direct treatment. Further studies are needed to improve and prove the autodissemination strategy under field conditions.

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